

Application No. 09/965,703  
Amendment dated April 20, 2005  
Reply to Office Action of November 24, 2004

**Amendments to Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

**Claim 1** (previously presented): A gene expression modulation system comprising:

- a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide encoding a first polypeptide comprising:
  - i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated;
  - ii) a ligand binding domain comprising a ligand binding domain from a nuclear receptor;
- b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide encoding a second polypeptide comprising:
  - i) a transactivation domain; and
  - ii) a ligand binding domain comprising a ligand binding domain from a nuclear receptor other than ultraspiracle (USP).

wherein the ligand binding domains from the first polypeptide and the second polypeptide are different.

**Claim 2** (original): The gene expression modulation system according to claim 1, further comprising a third gene expression cassette comprising:

- i) a response element to which the DNA-binding domain of the first polypeptide binds;
- ii) a promoter that is activated by the transactivation domain of the second polypeptide; and
- iii) the gene whose expression is to be modulated.

**Claim 3** (original): The gene expression modulation system according to claim 1, wherein the ligand binding domain of the first polypeptide is an ecdysone receptor polypeptide.

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**Claim 4 (original):** The gene expression modulation system according to claim 1, wherein the ligand binding domain of the second polypeptide is a retinoid X receptor polypeptide.

**Claim 5 (original):** A gene expression modulation system comprising:

- a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide encoding a first polypeptide comprising:
  - i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and
  - ii) a ligand binding domain comprising a ligand binding domain from an ecdysone receptor; and
- b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide encoding a second polypeptide comprising:
  - i) a transactivation domain; and
  - ii) a ligand binding domain comprising a ligand binding domain from a retinoid X receptor.

**Claim 6 (original):** The gene expression modulation system according to claim 5, further comprising a third gene expression cassette comprising:

- i) a response element to which the DNA-binding domain of the first polypeptide binds;
- ii) a promoter that is activated by the transactivation domain of the second polypeptide; and
- iii) the gene whose expression is to be modulated.

**Claim 7 (currently amended):** The gene expression modulation system according to claim 5, wherein the ligand binding domain of the first polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.

**Claim 8 (withdrawn):** The gene expression modulation system according to claim 5, wherein the ligand binding domain of the first polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID

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NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

**Claim 9 (withdrawn):** The gene expression modulation system according to claim 5, wherein the ligand binding domain of the second polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30.

**Claim 10 (withdrawn):** The gene expression modulation system according to claim 5, wherein the ligand binding domain of the second polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40.

**Claim 11 (original):** A gene expression modulation system comprising:

- a) a first gene expression cassette that is capable of being expressed in a host cell comprising a polynucleotide encoding a first polypeptide comprising:
  - i) a DNA-binding domain that recognizes a response element associated with a gene whose expression is to be modulated; and
  - ii) a ligand binding domain comprising a ligand binding domain from a retinoid X receptor; and
- b) a second gene expression cassette that is capable of being expressed in the host cell comprising a polynucleotide encoding a second polypeptide comprising:
  - i) a transactivation domain; and
  - ii) a ligand binding domain comprising a ligand binding domain from an ecdysone receptor.

**Claim 12 (original):** The gene expression modulation system according to claim 11, further comprising a third gene expression cassette comprising:

- i) a response element to which the DNA-binding domain of the first polypeptide binds;
- ii) a promoter that is activated by the transactivation domain of the second polypeptide; and
- iii) the gene whose expression is to be modulated.

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**Claim 13 (withdrawn):** The gene expression modulation system according to claim 11, wherein the ligand binding domain of the first polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30.

**Claim 14 (withdrawn):** The gene expression modulation system according to claim 11, wherein the ligand binding domain of the first polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40.

**Claim 15 (currently amended):** The gene expression modulation system according to claim 11, wherein the ligand binding domain of the second polypeptide is encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of ~~SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, and SEQ ID NO: 10.~~

**Claim 16 (withdrawn):** The gene expression modulation system according to claim 11, wherein the ligand binding domain of the second polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

**Claim 17 (withdrawn):** A gene expression cassette comprising a polynucleotide encoding a hybrid polypeptide comprising a DNA-binding domain or a transactivation domain, and an ecdysone receptor ligand binding domain, wherein the DNA binding domain or the transactivation domain is from a nuclear receptor other than an ecdysone receptor.

**Claim 18 (withdrawn):** A gene expression cassette comprising a polynucleotide encoding a hybrid polypeptide comprising a DNA-binding domain or a transactivation domain, and a retinoid X receptor ligand binding domain, wherein the DNA binding domain or the transactivation domain is from a nuclear receptor other than a retinoid X receptor.

**Claim 19 (withdrawn):** A gene expression cassette comprising a polynucleotide encoding a hybrid polypeptide comprising either a) a DNA-binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of

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SEQ ID NO: 41, SEQ ID NO: 43, and SEQ ID NO: 45, or b) a transactivation domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, and SEQ ID NO: 53, and a ligand binding domain encoded by a polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30.

**Claim 20 (withdrawn):** A gene expression cassette comprising a polynucleotide encoding a hybrid polypeptide comprising either a) a DNA-binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 42, SEQ ID NO: 44, and SEQ ID NO: 46, or b) a transactivation domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, and SEQ ID NO: 54, and a ligand binding domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40.

**Claim 21 (withdrawn):** An isolated polynucleotide encoding an ecdysone receptor polypeptide or a retinoid X receptor polypeptide comprising a truncation mutation, wherein the truncation mutation reduces ligand binding activity of the ecdysone receptor polypeptide or the retinoid X receptor polypeptide.

**Claim 22 (withdrawn):** An isolated polynucleotide encoding an ecdysone receptor polypeptide or a retinoid X receptor polypeptide comprising a truncation mutation, wherein the truncation mutation enhances ligand binding activity of the ecdysone receptor polypeptide or the retinoid X receptor polypeptide.

**Claim 23 (withdrawn):** An isolated polynucleotide encoding a retinoid X receptor polypeptide comprising a truncation mutation, wherein the truncation mutation increases ligand sensitivity of the retinoid X receptor polypeptide.

**Claim 24 (withdrawn):** An isolated polynucleotide encoding a retinoid X receptor polypeptide comprising a truncation mutation, wherein the truncation mutation

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increases ligand sensitivity of a heterodimer, wherein the heterodimer comprises said retinoid X receptor polypeptide and a dimerization partner.

**Claim 25 (withdrawn):** The isolated polynucleotide according to claim 24, wherein the dimerization partner is an ecdysone receptor polypeptide.

**Claim 26 (withdrawn):** An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, and SEQ ID NO: 30.

**Claim 27 (withdrawn):** An isolated polypeptide encoded by the isolated polynucleotide according to claim 26.

**Claim 28 (withdrawn):** An isolated truncated ecdysone receptor polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, and SEQ ID NO: 20.

**Claim 29 (withdrawn):** An isolated truncated retinoid X receptor polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 31, SEQ ID NO: 32, SEQ ID NO: 33, SEQ ID NO: 34, SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38, SEQ ID NO: 39, and SEQ ID NO: 40.

**Claim 30 (withdrawn):** A method of modulating the expression of a gene in a host cell comprising the gene to be modulated comprising the steps of:

- a) introducing into the host cell the gene expression modulation system according to claim 1; and
- b) introducing into the host cell a ligand that independently combines with the ligand binding domains of the first polypeptide and the second polypeptide;

wherein the gene to be expressed is a component of a chimeric gene comprising:

- i) a response element to which the DNA binding domain from the first polypeptide binds;
- ii) a promoter that is activated by the transactivation domain of the second polypeptide; and
- iii) a gene whose expression is to be modulated,

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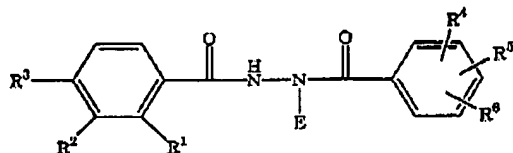
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whereby expression of the gene is modulated in the host cell.

**Claim 31 (withdrawn):** The method according to claim 30, wherein the ligand is either

a) a compound of the formula:



wherein:

E is a (C<sub>4</sub>-C<sub>6</sub>)alkyl containing a tertiary carbon or a cyano(C<sub>3</sub>-C<sub>5</sub>)alkyl containing a tertiary carbon;

R<sup>1</sup> is H, Me, Et, i-Pr, F, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, OH, OMe, OEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, SCN, or SCHF<sub>2</sub>;

R<sup>2</sup> is H, Me, Et, n-Pr, i-Pr, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CH<sub>2</sub>OMe, CH<sub>2</sub>CN, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, Ac, F, Cl, OH, OMe, OEt, O-n-Pr, OAc, NMe<sub>2</sub>, NEt<sub>2</sub>, SMe, SEt, SOCF<sub>3</sub>, OCF<sub>2</sub>CF<sub>2</sub>H, COEt, cyclopropyl, CF<sub>2</sub>CF<sub>3</sub>, CH=CHCN, allyl, azido, OCF<sub>3</sub>, OCHF<sub>2</sub>, O-i-Pr, SCN, SCHF<sub>2</sub>, SMe, NH-CN, or joined with R<sup>3</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R<sup>3</sup> is H, Et, or joined with R<sup>2</sup> and the phenyl carbons to which R<sup>2</sup> and R<sup>3</sup> are attached to form an ethylenedioxy, a dihydrofuryl ring with the oxygen adjacent to a phenyl carbon, or a dihydropyryl ring with the oxygen adjacent to a phenyl carbon;

R<sup>4</sup>, R<sup>5</sup>, and R<sup>6</sup> are independently H, Me, Et, F, Cl, Br, formyl, CF<sub>3</sub>, CHF<sub>2</sub>, CHCl<sub>2</sub>, CH<sub>2</sub>F, CH<sub>2</sub>Cl, CH<sub>2</sub>OH, CN, C<sup>o</sup>CH, 1-propynyl, 2-propynyl, vinyl, OMe, OEt, SMe, or SEt, or

b) an ecdysone, 20-hydroxyecdysone, ponasterone A, muristerone A, an oxysterol, a 22(R) hydroxycholesterol, 24(S) hydroxycholesterol, 25-epoxycholesterol, T0901317, 5- $\alpha$ -6- $\alpha$ -epoxycholesterol-3-sulfate (ECHS), 7-ketocholesterol-3-sulfate, farnesol, a bile acid, a 1,1-biphosphonate ester, or Juvenile hormone III.

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**Claim 32 (withdrawn):** The method according to claim 31, wherein the method further comprises a second ligand, wherein the second ligand is 9-cis-retinoic acid or a synthetic analog of retinoic acid.

**Claim 33 (withdrawn):** An isolated host cell into which the gene expression modulation system according to claim 1 has been introduced.

**Claim 34 (withdrawn):** The isolated host cell according to claim 33, wherein the host cell is selected from the group consisting of a bacterial cell, a fungal cell, a yeast cell, a nematode cell, an insect cell, a fish cell, a plant cell, an avian cell, an animal cell, and a mammalian cell.

**Claim 35 (withdrawn):** A non-human organism comprising a host cell into which the gene expression modulation system according to claim 1 has been introduced.

**Claim 36 (withdrawn):** The non-human organism according to claim 35, wherein the non-human organism is selected from the group consisting of a bacterium, a fungus, a yeast, a nematode, an insect, a fish, a plant, a bird, an animal, and a mammal.



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